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Localization of a *KEM1::lacZ* Fusion Protein in Yeast Cells

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***KEM1* is known to control the spindle pole body or microtubule function, probably in response to the cellular nutritional conditions in *Saccharomyces cerevisiae*. Transposon insertions were performed in the cloned *KEM1* gene using mini-Tn10-LUK element carrying *E. coli* β-galactosidase structural gene. A collection of random Tn10-LUK insertions defined an approximately 3.5 kb region required for the *KEM1* function. From this collection functional *KEM1::lacZ* protein fusions were identified. Indirect immunofluorescence using anti-β-galacatosidase antibodies localized the *KEM1::lacz* fusion protein to the periphery of the nucleus.**

KEY WORDS□spindle pole body, microtubules, *KEM1::lacZ*, intracellular localization

During conjugation in yeast *Saccharomyces cerevisiae*, nuclear fusion occurs immediately after cell fusion. Serial thin sections examined by electron microscopy suggest that the two nuclei are brought together by extranuclear microtubules emanating from the outer surface of the spindle pole body on the nuclear envelopes (6, 7). The first site of contact between the two nuclei appears to be at the position of the spindle pole bodies. Mutational analysis of the sequence of events leading to nuclear fusion has uncovered several genes required for the process. These include *KAR 1, KAR2, KAR3* (8), *KEM1* (15), *TUB2* (11, 23), and *BIK1* (2). Several studies with these genes demonstrate the critical involvement of microtubules and spindle pole body in nuclear fusion.

The *KEM1* gene was identified by *kem1* mutations which enhance the nuclear fusion defect of *kar1-1* (15). *KEM1* appears to control the spindle pole body or microtubule function, probably in response to the cellular nutritional conditions. This notion was based on the observations that the *kem1* mutatons cause defects in nuclear fusion during conjugation, mitotic chromosome transmission, spindle pole body duplication/separation during the cell division cycle, and response to nutritional conditions. In an attempt to identify and elucidate the functions of *KEM1* defined by mutational analysis, we have taken an alternative approach utilizing current molecular genetic techniques available in yeast. We expect that the information on the structure or intracellular localization of gene products would provide useful clues to the identification of gene functions.

The structure-function analysis has been greatly assisted by development of indirect immunofluorescence techniques in yeast. The arrangement of cytoskeletal proteins such as microtubules, actins, and intermediate filaments have been investigated by visualizing the structures with protein specific antibodies (1, 9, 14). Antibodies raised to the native proteins encoded by known genes have been very useful in revealing the intracellular localization of the gene products. β-galactosidase protein fusions often allow to localize the gene products with β-galactosidase specific antibodies, supporting the functions predicted for the gene products. In this paper, we will describe the insertion mutagenesis in *KEM1* for the construction of *KEM1::lacZ* fusion genes and the intracellular localization of the *KEM1* gene product.

MATERIALS AND METHODS

Strains, media, and genetic analysis

Yeast strain JK191 is *MATα, ura3-52, his4-29, cyh 2, kem1-5*. Strain JK147 is *MATb, α ura3-52, leu2-3, 112, trp1-1, ade2, cyh2*. Strain JK336 is *MATα, ura3-52, his4-29, kem1-1*. Yeast media were essentially as described by Sherman *et al.* (22). Benomyl plates were made by adding slowly the benomyl stock solution (10 mg/ml in dimethyl sulfoxide, stored at −20°C) to warm YEPD medium with vigorous swirling to prevent precipitation. Benomyl was a generous gift from E. I. Dupont deMours and Co., Inc. SC-Ura or SC-Ura-Leu

plates consisted of YNB with all amino acids added except uracil or except uracil and leucine.

Escherichia coli HB101 (4) was used for bacterial transformaton and plasmid growth. *E. coli* DB1329 is *recA56, arg*$^-$, *Δlac-proXIII, Nal*^{R}, *Rif*^{R}, *ara*$^-$/*F' lacI*^{q} (*pro*$^+$) and carries pNK629 (12). *Escherichia coli* DB1328 is a spontaneous λ-resistant mutant of HB101. Bacterial media were made as described by Maniatis *et al.* (18).

Transformation and DNA manipulation techniques

Yeast transformation was carried out by the lithium acetate method developed by Ito *et al.* (13) using 50 μg per transformation of sonicated calf thymus DNA (Sigma Inc.) as carrier. Transformants were plated on SC-Ura or Sc-Ura-Leu plates to select for plasmids. *E. coli* transformation was performed by the calcium chloride procedure of Mandel and Higa (17). Rapid isolation of plasmid DNA from *E. coli* was done by the boiling lysis method (10). Yeast DNA was prepared essentially same as described by Boeke *et al.* (3). Restriction endonuclease analysis and agarose gel electrophoresis were as described in Maniatis *et al.* (18).

Plasmid constructions

Plasmid pJI103 was constructed by inserting 5.5 Kb *Bam*HI-*Hin*dIII fragment carrying *KEM1* sequence between a *Bam*HI site and a *Hin*dIII site of YCp402. YCp402 (16) is a vector containing the *LEU2* marker and *CEN4*, which is derived from YCp50 by replacing *URA3* with *LEU2*. Plasmids pJI116 or pJI117 was constructed by isolating 9.5Kb *Bam*HI-*Hin*dIII fragment (contains *KEM1* sequence with a part of mini-Tn10-LUK sequence, *Hin*dIII cuts in *kanR* gene) from T67 or T79 and inserting between *Bam*HI and *Hin*dIII sites of YEp13. The construction of frameshift plasmid pJI126 was done by digesting pJI116 partially with *Nco*I, filling 3' recessed ends with Klenow, and ligating blunt ends. The deletion plasmid pJI128 was constructed by partial digestion of T67 with *Bgl*II and ligation.

Mini-Tn10-LUK transposon mutagenesis

Transposon mutagenesis was performed essentially same as described by Huismas *et al.* (12) except that we followed the protocol revised by Andy Hoyt. The *E. coli* strain DB1329 carrying pNK629 (containing Tn10 transposase with an IPTG inducible promoter) was transformed with a target plasmid pJI103 and transformants were selected on LB+Tet+Amp plates to maintain both pNK629(Tet^R) and pJI103(Amp^R). A transformant BJ80 was grown overnight in LB+Tet+Amp liquid medium at 37°C, diluted into LB+0.2 % maltose +1mM IPTG+Tet+Amp and grown to log phase (Klett 50). Forty milliliters of culture was centrifuged and resuspended in 4 ml of LB +maltose+IPTG+10 mM $MgCl_2$. λLUK lysates were added to an m.o.i. of 0.4, which was incubated at room temperature for 30 min to allow phage infection. This culture was diluted with 36 ml of LB+IPTG and incubated at 37°C for 1.5 hr. After cells were centrifuged and resuspended in LB, appropriate dilutions were made and plated on LB+Na pyrophosphate (1.25 mM)+kan (50 μg/ml)+Amp. Plates were incubated overnight at 39~42°C. We prepared DNA from a pool of about 10,000 colonies, which was transformed into the *E. coli* strain DB1328 selecting for Kan^R and Amp^R colonies.

Plate mating

Strains to be tested were patched on YEPD plates. Lawns of strains with an opposite mating type and complementary auxotrophic markers were cross replica-plated with plates containing patches of the strains to be tested onto fresh YEPD plates for a brief mating. After 4 hr incubation at 30°C (or 5 hr at 24°C or 3.5 hr at 34°C), the mating plates were replica-plated to minimal media to select for diploids.

A quick β-galactosidase assay

Cells were grown to log phase ($O.D._{600}$=1.0). 1 ml of culture was centrifuged, washed once with dH_2O, and resuspended in 0.8 ml of Z-buffer (19) supplemented with 0.0075% sodium dodecyl sulfate. Cells were permeabilized by adding 60 μl of chloroform followed by vigorous agitation. The assay was started by adding 0.2 ml of ONPG (4 mg/ml) at 28°C and stopped by adding 0.45 ml of 1 M Na_2CO_3. This reaction mixture was centrifuged and measured at $O.D._{420}$. Units of β-galactosidase are expressed by the formula (1,000 ×$O.D._{420}$)/($O.D._{600}$×volume of culture×minutes of assay).

Western blots

Total protein was isolated from yeast cells by the following procedure. 5 ml of cell culture grown to log phase ($O.D._{600}$=1.0) was centrifuged, washed once with dH_2O, and resuspended in 50 ml of dH_2O. Cells were lysed by agitation for 90 seconds on a vortex mixer in the presence of 10 μl of 100% trichloroacetic acid and 0.2 g of glass beads. 500 μl of cold 5% trichloroacetic acid was added to this mixture, which was centrifuged at 13 K for 20 minutes. The pellet was washed once with 1.5 ml of cold H_2O and centrifuged for 3-5 minutes. Pellets were resuspended in 150 μl of sample buffer (12.5% glycerol, 2% sodium dodecyl sulfate, 80 mM Tris-hydrochloride, pH 6.8, 2.5% β-mercaptoethanol, and 0.12% bromophenol blue), neutralized by adding 3 μl of 2 M Tris base, and boiled for 5 minutes. After centrifugation at room temperature, 25 μl of supernatant of each sample was loaded onto a 7.5% SDS-polyacrylamide gel, subjected to electrophoresis, and transferred to nitrocellulose filter (24). The filter was placed in a blocking solution (5% instant milk, 150 mM NaCl, 50 mM Tris hydrochloride, pH 7.5, 0.5% sodium azide, and 0.5% Tween20) for 30 minutes with shaking. β-galactosidase antibodies (gener-

ously given by Tom Mason) were added (1:10 dilution) and incubated for 3 hours. The filter was washed three times in blocking solution without milk and incubated in blocking solution with the [I^{125}]-protein A for 2 hrs. The filter was washed three times as above and exposed to X-ray film.

Immunofluorescence

Indirect immunofluorescence was performed by the method of Adams *et al.* (1) with a few modifications. 10 m*l* of yeast culture grown to early log phase (<1×10^7 cells/m*l*) was fixed by adding 1 m*l* of 1 M potassium phosphate, pH 6.5 (P buffer) and 1 m*l* of 37% formaldehyde. After incubation at room temperature for 2hrs, cells were centrifuged (4,000×g), washed twice with 0.1 M P buffer, washed once with 1.2 M sorbitol/0.1 M P buffer, and resuspended in 1 m*l* of 1.2 M sorbitol/0.1 M P buffer. 5 μl of β-mercaptoethanol and 30 μl of zymolyase (10 mg/m*l*, 60 K activity, Kirin Co.) were added to the cell suspension to digest off the cell wall. After 1.5 hr incubation at 30°C with gentle rocking, cells were centrifuged, washed once with 1.2 M sorbitol/0.1 M P buffer, and resuspended in 3 m*l* of 1.2 M sorbitol/0.1 M P buffer. 15 μl of cell suspension was applied to a well on a polylysine coated slide (8 well Flowlabs slide). The slide was immersed in cold methanol (−20°C) for 6 minutes and into cold acetone (−20°C) for 30 seconds. The antibody reaction was carried out by adding 15 μl of 1:40 dilution of β-galactosidase antiserum in BSA/sorbitol/P buffer (10 mg/m*l* BSA, 1.2 M sorbitol, 0.1 M potassium phosphate, pH 6.5) to each well and incubating in a moist chamber at room temperature for 1 hr. Antibodies were washed off three times with BSA/sorbitol/P buffer and secondary antibody reaction was carried out by adding 15 μl of Rhodamine-conjugated anti-mouse antibodies (1:500 dilution, Boehringer Mannheim Biochemicals) to each well. After incubation at room temperature in dark for 1 hr, antibodies were washed off four times with BSA/sorbitol/P buffer and twice with sorbitol/P buffer. Fifteen microliters of DAPI (1 μg/m*l*) was added for 5 minutes to stain nuclear DNA. After a wash with sorbitol/P buffer, slides were mounted with P-phenylenediamine/90% glycerol and examined on the fluorescent microscope.

RESULTS

Transposon insertions in *KEM1* using mini-Tn10-LUK

To make insertion mutations in the cloned *KEM1* gene, we performed transposon mutagenesis using mini-Tn10-LUK system developed by Huisman *et al.* (12). For the analysis of *KEM1*, this system has several useful features. The random sites and the high frequency of transposition events allow definition of the functional boundaries of the *KEM1* gene. The expression of the *lacZ* gene, possibly resulting from in-frame fusion with *KEM1* provides *KEM1::lacZ* protein fusions which can be used to localize the *KEM1* gene products.

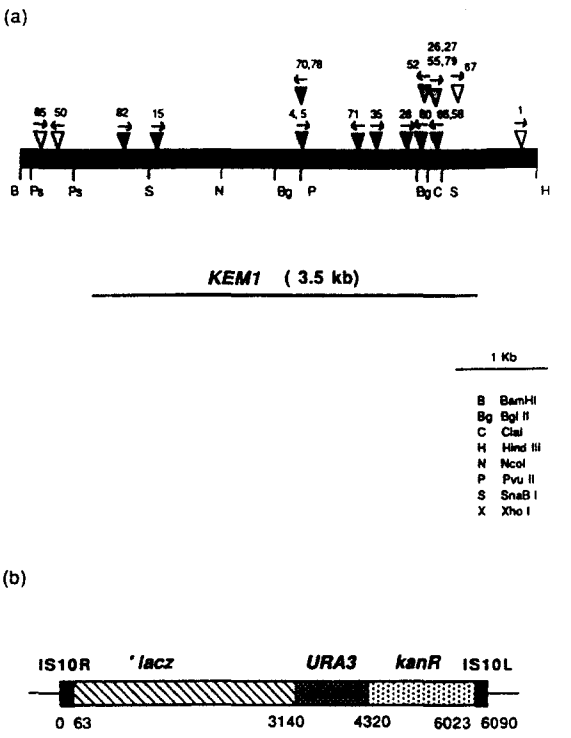

Fig. 1. *Restriction map of the plasmid vector used for transposon insertion analysis as well as the structure of mini-Tn10-LUK element.*
(a) The DNA insert of pJI103 is shown as a filled-in box. The 5.5kb *Bam*HI-*Hin*dIII fragment is inserted at the *Bam*HI and *Hin*dIII sites of YCp402. The site of insertion is indicated with a triangle. Numbers indicate the insertion plasmids. The fill of the triangle describes the complementing activity of the insertion plasmids; white (fully complementing activity), black (no complementing activity), gray (partially complementing activity). The arrow on top indicates the orientation of the mini-Tn10-LUK element from *lacZ* toward *KanR*. The predicted region of KEM1 function is shown.
(b) The structure of mini-Tn10-LUK element. Numbers represent the distance in nucleotide of each junction from the left end of the Tn 10-LUK element. *lacZ* transcription is from left to right. The *lacZ* structural gene segment begins at codon eight of the protein coding sequence.

Transposon insertions into the *KEM1* gene were isolated as follows. *E. coil* strain DB1329 harboring a plasmid that gives an IPTG-inducible production of Tn10 transposase function was transformed with the target plasmid pJI103 (Fig. 1) which contains a 5.5 Kb fragment of the *KEM1* gene on YCp402 (16). After induction of transposase function in the transformants BJ80, we introduced the mini-Tn10-LUK carried on λ-phage into this strain. Mini-Tn10-LUK consists of the two termini of Tn10, the *E. coli lacZ* gene, the *KanR* gene, and the yeast *URA3* gene (Fig. 1). Transposition of the mini-Tn10-LUK element into the plasmid pJI103 was selected on kanamycin plates. Plasmid DNAs were prepared from a pool of Kan^R colonies and introduced into an *E. coli* strain DB1328 by transformation for individual analysis. A total of 85 transformants were examined by restriction analysis after rapid plasmid preparations and all shown to have insertions. Of those, 43 plasmids contained insertions in the *KEM1* sequence, which were analyzed further for more precise restriction mapping. Fig. 1 demonstrates the structure of mini-Tn10-LUK and its insertion sites in the *KEM 1* gene with regard to the known restriction sites. The arrow indicates the orientation of mini-Tn10-LUK, directing from *lacZ* to *KanR*.

Insertion mutations define a 3.5 Kb region required for *KEM1* function

To test the complementing activity of each plasmid carrying a mini-Tn10-LUK insertion in *KEM1*, we transformed strain JK191 (*kem1-5*) with these plasmids. Patches of transformant strains were made and tested for benomyl hypersensitivity by replica-plating onto benomyl plates (10 or 15 μg/ml benomyl in YEPD medium). The Kem$^-$ phenotype was also examined by a plate mating assay. The master plates with patches of strains to be tested were replica-plated onto a lawn of *KAR1* cells and onto a lawn of *kar1-1* cells. After mating for 4hrs at 30°C, plates were replica-plated onto SC plates to select diploids.

The results are summarized in Table 1. The plasmid with insertion T50 has full *KEM1* complementing activity, indicating that T50 lies outside the *KEM1* gene boundary. The insertion T82 destroys the complementing activity, indicating that it is internal to the *KEM1* gene. Starting from T82, a continuous region of 3.5 Kb is shown to be required for *KEM1* function. Plasmids with insertions T27, T52, T55, or T79 are partially functional. They complement the benomyl-hypersensitive phenotype but not the Kem$^-$ phenotype. This partial activity implies that T27, T52, T55,and T79 lie internal to the *KEM1* gene and they define a boundary of *KEM1* function. Insertion T67 which lies right next to those insertions with partial activity (within 100 bps) shows full complementing activity. The conclusion from these results is that a 3.5 Kb region defined by insertion mutations is required for KEM1 function.

Table 1. *Complementing activity of transposon insertion plasmids.*

Plasmid #[a]	Benomyl-sensitivity[b]	Diploid formation[c]	β-galactosidase activity[d]
T85	+	+	−
T50	+	+	−
T82	−	−	−
T15	+	+	−
T4, 5, 38, 39, 49, 63	−	−	−
T70, 78	−	−	−
T71	−	−	−
T35	−	−	−
T28	−	−	−
T80	−	−	−
T52	+/−	+/−	−
T26	+	+	+
T27	+/−	−	+
T55	++/−	−	+
T79	++/−	−	+
T58, 66	−	−	−
T67	+	+	+
T1	+	+	−

[a] K191 (*kem1-5*) was transformed with the plasmids indicated.

[b] Complementation of benomyl-hypersensitivity of *kem1-5* is indicated. +, full complementation; ++/− and +/−, partial complementation; −, no complementation.

[c] Complementation of Kem$^-$-phenotype (diploid formation in crosses to a *kar1-1* strain) of *kem1-5* is indicated. +, full complementation; −, no complementation.

[d] β-galactosidase activity determined by using X-gal indicator plates. +, blue patches; −, white patches.

***KEM1::lacZ* protein fusion**

The *lacZ* gene of mini-Tn10-LUK element (Fig. 1) lacks the first seven amino acids and both transcriptional and translational initiation signals. Therefore, insertion in the proper orientation and reading frame within the target gene will create a protein fusion. This fusion extends from the target gene, across the 70 bp terminus of the transposon, and into the *lacZ* gene. To identify *KEM1::lacZ* fusion plasmids, we tested the strains haboring plasmids with mini-Tn10-LUK insertions in *KEM1* for β-galactosidase activity on X-Gal indicator plates. Five plasmids (T26, T27, T55, T79, and T67) gave blue colonies, indicating efficient production of β-galactosidase. This result was confirmed by β-galactosidase assay using chloroform-permeabilized cells. As shown in Fig. 1, all of five insertions showing β-galactosidase

activity are in the same orientation, strongly suggesting that reading frame of the *KEM1* gene proceeds in the direction from a *Pst*I site toward a *Hin*dIII site. If this is the correct direction of the reading frame, insertions T26, T27, T55, T79, and T67 are located at the C-terminus of the *KEM 1* polypeptide. These locations probably explain full *KEM1* complementing activity of plasmids T 26 and T67 and partial activity of T27, T55, and T79. A frame shift mutation at the upstream *Nco*I site in the *KEM1* sequence (pJI126) destroys the *KEM1* complementing activity as well as β-galactosidase activity of plasmid T67. These results suggest that in-frame insertion T67 is internal to a coding sequence of the *KEM1* gene and creates a functional *KEM1::lacZ* fusion.

To demonstrate that in-frame insertion of mini-Tn10-LUK in the *KEM1* gene generates the expected *KEM1::lacZ* fusion protein, we peformed western blot analysis using antibodies to β-galactosidase. Extracts of yeast cells carrying different plasmids were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose filter, and hybrid proteins were identified by β-galactosidase antibodies and [I^{125}]-lableled protein A. Fusions T67 and T79 produce hybrid proteins with approximate molecular weight of 230 Kd (lane 67, 79 in Fig. 2), which is expected from the location of these insertions at the C-terminus of *KEM1*. In cells with plasmids pJI116 and pJI117 (insertion T67 and T79 on 2 μ high copy plasmid) hybrid proteins were produced at the increased level (Fig. 2). In-frame deletion of a 1.6 Kb *Bgl*II fragment in the *KEM1* sequence of fusion T67 (pJI128) reduces the molecular weight of hybrid proteins by the size of the deleted fragment (lane 128 in Fig. 2). pJI128 is nonfunctional in complementation of *kem1* mutation . The plasmid pJI126 carrying a frame shift mutation at the upstream *Nco*I site does not produce hybrid proteins (lane 126 in Fig. 2). These results confirm the conclusion that in-frame insertions of mini-Tn10-LUK in *KEM1* sequence generated funtional *KEM1::lacZ* protein fusions.

Localization of *KEM::lacZ* protein fusions

To determine the cellular location of the *KEM1* gene product, we performed immunofluorescence microscopy on cells carrying a *KEM1::lacZ* functional protein fusion on a high copy vector. Formaldhyde-fixed cells were treated with anti-β-galactosidase antibody which was visualized using rhodamine-conjugated antibodies. The *KEM1::lacZ* fusion gives bright staining around the nucleus (Fig. 3, panels A-F) whereas a *HTS1::lacZ* fusion (generously given by Chiu, personal communication) as a cytoplasmic control stains the whole cytoplasm (panels G-I). The *HTS1* gene encodes the histidine-tRNA synthetase (20). Cells treated with mating pheromones or zygotes in mating culture also show staining around the nucleus (data not shown). These data suggest that the functional *KEM1::lacZ* fusion proteins are localized to the nuclear periphery.

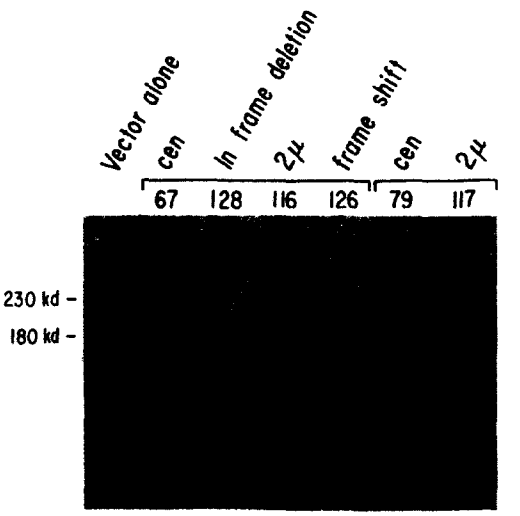

Fig. 2. *Western blot analysis of KEM1::lacZ fusion proteins.*
The yeast strain JK147 was transformed with various plasmids carrying *KEM1::lacZ* protein fusions. Protein extracts were prepared from whole cells, resolved on 7.5% SDS-polyacrylamide gel, and transferred onto nitrocellulose filter. Fusion proteins were identified by using anti-β-galactosidase antibodies and [I^{125}]-protein A. Lane 67, plasmid T67 carrying *KEM1::lacZ* fusion on a Cen-vector; lane 128, plasmid pJI128 carrying *KEM1::lacZ* fusion with 1.6 kb *Bgl*II in-frame deletion in *KEM1*; lane 116, plasmid pJI116 carrying *KEM1::lacZ* on a 2 μ-vector; lane 126, plasmid pJI126 carrying *KEM1::lacZ* fusion with a *Nco*I site filled generating a frame shift mutation in *KEM1*; lane 79, plasmid T79 carrying *KEM1::lacZ* fusion on a Cen-vector; lane 117, plasmid pJI117 carrying *KEM1::lacZ* on a 2 μ-vector.

DISCUSSION

In this paper, we have described transposon insertion analysis of *KEM1* and intracellular localization of a *KEM1::lacZ* hybrid protein. A collection of random insertions of mini-Tn10-LUK element (12) in *KEM1* defined an approximately 3.5 Kb region required for the *KEM1* function and gave functional *KEM1::lacZ* protein fusions. Indirect immunofluorescence using anti-β-galactosidase antibodies localized the *KEM1::lacZ* hybrid protein to the nuclear periphery.

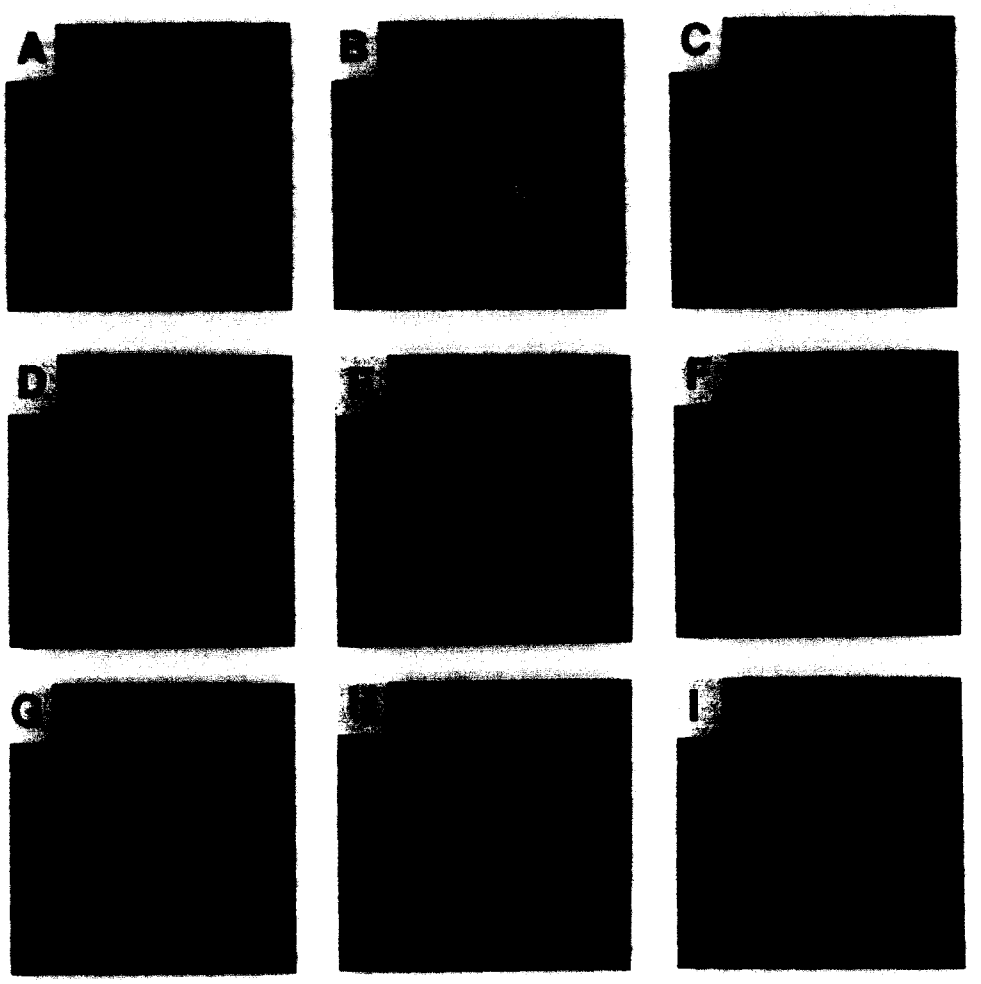

Fig. 3. *Intracellular localization of KEM1::lacZ fusion proteins revealed by indirect immunofluorescence.*
The yeast strain JK147 was transformed with plasmids carrying *KEM1::lacZ* or *HTS1::lacZ* protein fusions. Each set of three panels represents the same cell revealed by (1) anti-β-galactosidase staining, (2) DAPI staining, and (3) Nomarsky optics.
A-F cells carrying *KEM1::lacZ* fusion on a 2 μ-vector (pJI116); G-I cells carrying *HTS1::lacZ* fusion on a 2 μ-vector (pIC 231).

The cellular location of the *KEM1::lacZ* fusion protein at the nuclear periphery suggests that the native *KEM1* proteins are localized to the same region. The nucleus of a eucaryotic cell is enclosed by a double membrane structure called nuclear envelope (5). Inner and outer membranes of the nuclear envelope are separated by the perinuclear space. The outer membrane is continuous with the endoplasmic reticulum (ER) membrane. In indirect immunofluorescence microscopy with *KEM1::lacZ*, the staining around the nucleus was bright and faded out into the cytoplasm suggesting that these hybrid proteins are presumably present in the nuclear envelope and may be in ER membranes as well. The *KEM1* gene is known to affect nuclear fusion during conjugation as well as spindle pole body function. Nuclear fusion would require the fusion of two nuclear envelopes followed by the reorganization of membrane structure. Although the mechanism by which membranes fuse is not understood, it is very likely that a majority of the participating proteins are present at or near the nucleus. The spindle pole body that is also affected by *KEM1* is embedded in the nuclear envelope participating in nuclear fusion processes as well as in organization of microtubules (6, 7, 21). The functions of the *KEM1* gene defined by

mutational analysis appear to be in good agreement with the predicted cellular location of the KEM1 proteins.

Studies on the cellular localization of the gene products are often hindered by a number of reasons. An approach using indirect immunofluorescence requires an abundance of stable proteins in the cell for visualization in the light microscope. Efficient and stable antibody-antigen interaction is another critical factor. The use of anti-β-galactosidase antibodies to stain the β-galactosidase hybrid proteins often gives an informative signal which the antibodies against native proteins can not. However, the large size of β-galactosidase proteins may lead to mislocalization. We have tried to use a functional *KEM1::lacZ* protein fusion on a high-copy vector. The *KEM1* on a high-copy vector does not seem to affect either mitotic growth nor the diploid formation on mating. The investigation on the cellular localization of the *KEM1* proteins should be checked by using *KEM1*-specific antibodies and cell fractionation techniques to verify the results with *KEM1::lacZ* protein fusions.

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초록: 효모세포에서 *KEM1::lacZ* 융합 단백질의 위치결정

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*Saccharomyces cerevisiae*의 *KEM1* 유전자는 세포의 영양 상태에 따라 spindle pole body나 microtubules의 기능을 조절하는 것으로 알려져 있다. 이 유전자 산물의 세포내 분포 및 기능을 규명하기 위하여, *KEM1::lacZ* 융합 유전자를 제조하였다. 즉, 클론된 *KEM1* 유전자에 대장균의 β-galactosidase 구조유전자를 갖는 mini-Tn10-LUK element를 무작위 삽입한 pool을 제조하고, 이를 분석하여 *KEM1*의 기능 부위가 약 3.5 kb에 해당함을 확인하였고, *KEM1*의 기능이 살아있는 *KEM1::lacZ* 융합 유전자의 클론을 선별하였다. 이 클론을 β-galactosidase 항체를 이용한 indirect immunofluorescence 방법으로 분석하여 *KEM1::lacZ* 융합 단백질이 핵주변에 위치함을 확인하였다.